(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



(43) International Publication Date 10 September 2004 (10.09.2004)

PCT

(10) International Publication Number WO 2004/076638 A2

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(51) International Patent Classification7:

C12N

(21) International Application Number:

PCT/US2004/005654

(22) International Filing Date: 25 February 2004 (25.02.2004)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

Filed on

10/374,780 10/675,852

25 February 2003 (25.02.2003) US

30 September 2003 (30.09.2003)

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(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:

US

10/374,780 (CIP) 25 February 2003 (25.02.2003)

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425 Market Street, San Francisco, CA 94105-2482 (US). (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

19460 (US).

of inventorship (Rule 4.17(iv)) for US only

Published:

without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYNUCLEOTIDES AND POLYPEPTIDES IN PLANTS

(57) Abstract: The invention relates to plant transcription factor polypeptides, polynucleotides that encode them, homologs from a variety of plant species, and methods of using the polynucleotides and polypeptides to produce transgenic plants having advantageous properties compared to a reference plant. Sequence information related to these polynucleotides and polypeptides can also be used in bioinformatic search methods is also disclosed.



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sequence shown in the Sequence Listing due to degeneracy in the genetic code, are also within the scope of the invention.

Altered polynucleotide sequences encoding polypeptides include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide encoding a polypeptide with at least one functional characteristic of the instant polypeptides. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding the instant polypeptides, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding the instant polypeptides.

Allelic variant refers to any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (i.e., no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene. Splice variant refers to alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein encoded by a splice variant of an mRNA transcribed from a gene.

Those skilled in the art would recognize that, for example, G481, SEQ ID NO: 88, represents a single transcription factor; allelic variation and alternative splicing may be expected to occur. Allelic variants of SEQ ID NO: 87 can be cloned by probing cDNA or genomic libraries from different individual organisms according to standard procedures. Allelic variants of the DNA sequence shown in SEQ ID NO: 87, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of SEQ ID NO: 88. cDNAs generated from alternatively spliced mRNAs, which retain the properties of the transcription factor are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individual organisms or tissues according to standard procedures known in the art (see USPN 6,388,064).

Thus, in addition to the sequences set forth in the Sequence Listing, the invention also encompasses related nucleic acid molecules that include allelic or splice variants of SEQ ID NO: 2N - 1, wherein N = 1- 229, SEQ ID NO: 459-466; 468-487; 491-500; 504; 506-511; 516-520; 523-524; 527; 529; 531-533; 538-539; 541-557; 560-568; 570-586; 595-596; 598-606; 610-620; 627-634; 640-664; 670-707; 714-719; 722-735; 740-741; 743-779; 808-823; 825-834; 838-850; 855-864; 868-889; 892-902; 908-909; 914-921; 924-925; 927-932; 935-942; 944-952; 961-965; 968-986; 989-993; 995-1010; 1012-1034; 1043-1063; 1074-1080; 1091-1104; 1111-1121; 1123-1128; 1134-1138; 1142-1156; 1159-1175;

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1187-1190; 1192-1199; 1202-1220; 1249-1253; 1258-1262; 1264-1269; 1271-1287; 1292-1301; 1303-1309; 1315-1323; 1328-1337; 1340-1341; 1344-1361; 1365-1377; 1379-1390; 1393-1394; 1396-1398; 1419-1432; 1434-1452; 1455-1456; 1460-1465; 1468-1491; 1499; 1502; 1505-1521; 1523-1527; 1529-1532; 1536-1539; 1542-1562; 1567-1571; 1573-1582; 1587-1592; 1595-1620; 1625-1644; 1647-1654; 5 1659-1669; 1671-1673; 1675-1680; 1682-1686; 1688-1700; 1706-1709; 1714-1726; 1728-1734; 1738-1742; 1744-1753; 1757-1760; 1763-1764; 1766-1768; 1770-1780; 1782-1784; 1786-1789; 1791-1804; 1806-1812; 1814-1837; 1847-1856; 1858-1862; 1864-1873; 1876-1882; 1885-1896; 1902-1910; 1913-1916; 1921-1928; 1931-1936; 1940-1941; 1944-1946, 2907-2941, 2944, 2945, 2947, 2949, or SEO ID NO: 2N - 1, wherein N = 974-1101, and include sequences which are complementary to any of the 10 above nucleotide sequences. Related nucleic acid molecules also include nucleotide sequences encoding a polypeptide comprising or consisting essentially of a substitution, modification, addition and/or deletion of one or more amino acid residues compared to the polypeptide as set forth in any of SEQ ID NO: 2N, wherein N = 1-229, SEQ ID NO: 467; 488-490; 501-503; 505; 512-515; 521-522; 525-526; 528; 530; 534-537; 540; 558-559; 569; 587-594; 597; 607-609; 621-626; 635-639; 665-669; 708-713; 15 720-721; 736-739; 742; 780-807; 824; 835-837; 851-854; 865-867; 890-891; 903-907; 910-913; 922-923; 926; 933-934; 943; 953-960; 966-967; 987-988; 994; 1011; 1035-1042; 1064-1073; 1081-1090; 1105-1110; 1122; 1129-1133; 1139-1141; 1157-1158; 1176-1186; 1191; 1200-1201; 1221-1248; 1254-1257; 1263; 1270; 1288-1291; 1302; 1310-1314; 1324-1327; 1338-1339; 1342-1343; 1362-1364; 1378; 1391-1392; 1395; 1399-1418; 1433; 1453-1454; 1457-1459; 1466-1467; 1492-1498; 1500-1501; 1503-20 1504; 1522; 1528; 1533-1535; 1540-1541; 1563-1566; 1572; 1583-1586; 1593-1594; 1621-1624; 1645-1646; 1655-1658; 1670; 1674; 1681; 1687; 1701-1705; 1710-1713; 1727; 1735-1737; 1743; 1754-1756; 1761-1762; 1765; 1769; 1781; 1785; 1790; 1805; 1813; 1838-1846; 1857; 1863; 1874-1875; 1883-1884; 1897-1901; 1911-1912; 1917-1920; 1929-1930; 1937-1939; 1942-1943; 2942 or 2943, 2945, 2947, 2949, or SEQ ID NO: 2N, wherein N = 974-1101. Such related polypeptides may comprise, for example, additions and/or deletions of one or more N-linked or O-linked glycosylation sites, or an addition and/or 25 a deletion of one or more cysteine residues.

For example, Table 4 illustrates, e.g., that the codons AGC, AGT, TCA, TCC, TCG, and TCT all encode the same amino acid: serine. Accordingly, at each position in the sequence where there is a codon encoding serine, any of the above trinucleotide sequences can be used without altering the encoded polypeptide.

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251	G1266	OE	Increased tolerance to disease	Increased tolerance to Erysiphe
251 1200 0		OL	Growth regulation; nutrient	Altered C/N sensing
			uptake	Altered O'IV sensing
253 ·	G1275	OE	Altered architecture	Reduced apical dominance
255	G1305	OE	Increased tolerance to abiotic stress	Reduced chlorosis in heat
257	G1322	OE	Increased tolerance to abiotic	Increased seedling vigor in cold
			stress	Reduced size
			Altered size	Increase in M39480
			Leaf glucosinolates	Constitutive photomorphogenesis
	i .		Altered light response and/or	Altered C/N sensing: increased
			shade tolerance	tolerance to low nitrogen
			Growth regulation; nutrient	
			uptake	
259	G1323	OE	Altered seed oil	Decreased seed oil
			Altered seed protein	Increased seed protein
261	G1330	OE	Altered hormone sensitivity	Ethylene insensitive when germinated
				in the dark on ACC
263	G1331	OE	Altered light response and/or	Constitutive photomorphogenesis
			shade tolerance	Altered C/N sensing
			Growth regulation; nutrient	
		<u>.</u>	uptake	
265	G1332	OE	Altered trichomes	Reduced trichome density
			Growth regulation; nutrient	Altered C/N sensing
			uptake	
267			Increased tolerance to disease	Increased tolerance to Fusarium
269	G1411	OE	Altered architecture	Loss of apical dominance
2607	G1412	KO	Altered light response and/or	Increased shade tolerance; lack of shade
			shade tolerance	avoidance phenotype
271	G1417	KO	Altered seed oil	Increase in 18:2, decrease in 18:3 fatty
		-		acids
273			Altered seed protein	Increased seed protein
275			Altered flower	Altered flower structure
277	G1451		Altered size	Increased plant size
	ŀ		Altered leaf	Large leaf size
	<u> </u>		Altered seed oil	Altered seed oil content
279	G1452	OE	Altered trichomes	Reduced trichome density
			Altered leaf	Altered leaf shape, dark green color
	1		Altered hormone sensitivity	Reduced sensitivity to ABA
			Altered flowering time	Better germination on sucrose, salt
			Increased tolerance to abiotic	Late flowering
001	01460	-	and osmotic stress	Increased tolerance to drought
281	G1463		Altered senescence	Premature senescence
283			Altered seed oil	Increased seed oil content
285	G1478	OE	Altered seed protein	Decreased seed protein content
			Altered flowering time	Late flowering
	1	-	Altered seed oil	Increased seed oil content
287	G1482		Altered pigment	Increased anthocyanins
			Altered root	Increased root growth
289	G1488	OE	Altered seed protein	Altered seed protein content
			Altered light response and/or	Constitutive photomorphogenesis
]	shade tolerance	Reduced apical dominance, shorter
	ļ	<u> </u>	Altered architecture	stems
291	161404	LOE	Altered flowering time	Early flowering

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			indeterminate growth
	Altered shoot meristem development Stem bifurcations:	G390; G391	Ornamental modification of plant architecture, manipulation of growth and development, increase in leaf numbers, modulation of branching patterns to provide improved yield or biomass
	Altered branching pattern	G427; G568; G988; G1543; G1794	Ornamental modification of plant architecture, improved lodging resistance
Ĭ.	Apical dominance Reduced apical dominance:	G47; G211; G1255; G1275; G1411; G1488; G1794; G2509	Omamental modification of plant architecture
		•	Ornamental modification of plant architecture, increased plant product (e.g., diterpenes, cotton) productivity, insect and herbivore resistance
	Increase in trichome number, size or density:	G362; G634; G838; G2838	~
	Stem morphology and altered vascular tissue structure	G47; G438; G748; G988; G1488	Modulation of lignin content; improvement of wood, palatability of fruits and vegetables
	Root development Increased root growth and proliferation: Increased root hairs:		Improved yield, stress tolerance; anchorage
	Altered seed development, ripening and germination	G979	·
	Cell differentiation and cell proliferation	G1540	Increase in carpel or fruit development; improve regeneration

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G779, G988, G1075, G1140, G1499, G1947, G2143, G2557 and their functional equivalogs, possess reduced fertility; flowers are infertile and fail to yield seed. These could be desirable traits, as low fertility could be exploited to prevent or minimize the escape of the pollen of genetically modified organisms (GMOs) into the environment.

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The alterations in shoot architecture seen in the lines transformed with G47, G1063, G1645, G2143, and their functional equivalogs indicates that these genes and their equivalogs can be used to manipulate inflorescence branching patterns. This could influence yield and offer the potential for more effective harvesting techniques. For example, a "self pruning" mutation of tomato results in a determinate growth pattern and facilitates mechanical harvesting (Pnueli et al. (2001) Plant Cell 13(12): 2687-702).

One interesting application for manipulation of flower structure, for example, by introduced transcription factors could be in the increased production of edible flowers or flower parts, including saffron, which is derived from the stigmas of Crocus sativus.

Genes that later silique conformation in brassicates may be used to modify fruit ripening processes in brassicates and other plants, which may positively affect seed or fruit quality.

A number of the presently disclosed transcription factors may affect the timing of phase changes in plants. Since the timing or phase changes generally affects a plant's eventual size, these genes may prove beneficial by providing means for improving yield and biomass.

General development and morphology: shoot meristem and branching patterns. Several of the 20 presently disclosed transcription factor genes, including G390 and G391, and G1794, when introduced into plants, have been shown to cause stem bifurcations in developing shoots in which the shoot meristems split to form two or three separate shoots. These transcription factors and their functional equivalogs may thus be used to manipulate branching. This would provide a unique appearance, which may be desirable in ornamental applications, and may be used to modify lateral branching for use in the forestry industry. A reduction in the formation of lateral branches could reduce knot formation. Conversely, increasing the number of lateral branches could provide utility when a plant is used as a view- or windscreen.

General development and morphology: apical dominance: The modified expression of presently disclosed transcription factors (e.g., G47, G211, G1255, G1275, G1411, G1488, G1794, G2509 and their equivalogs) that reduce apical dominance could be used in ornamental horticulture, for example, to modify plant architecture, for example, to produce a shorter, more bushy stature than wild type. The latter form would have ornamental utility as well as provide increased resistance to lodging.

General development and morphology: trichome density, development or structure. Several of the presently disclosed transcription factor genes have been used to modify trichome number, density, trichome cell fate, amount of trichome products produced by plants, or produce ectopic trichome

SEQUENCE LISTING

<110>	Sherman, Bradley K Riechmann, Jose Luis Jiang, Cai-Zhong Heard, Jacqueline E Haake, Volker Creelman, Robert A Ratcliffe, Oliver Adam, Luc J Reuber, T. Lynne Keddie, James Dubell III, Arnold N Pineda, Omaira Repetti, Peter Century, Karen Gutterson, Neal Yu, Guo-Liang Broun, Pierre E Kumimoto, Roderick W Pilgrim, Marsha L						
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- Cys Phe Ser His Pro Glu Ser Pro Ser Thr Lys Thr Tyr Val Ala Ala 65 70 75 80
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- Ile Lys Gly Arg Val Ala Phe Lys Thr Arg Ser Glu Val Glu Val Leu 100 105 110
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